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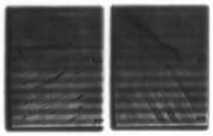

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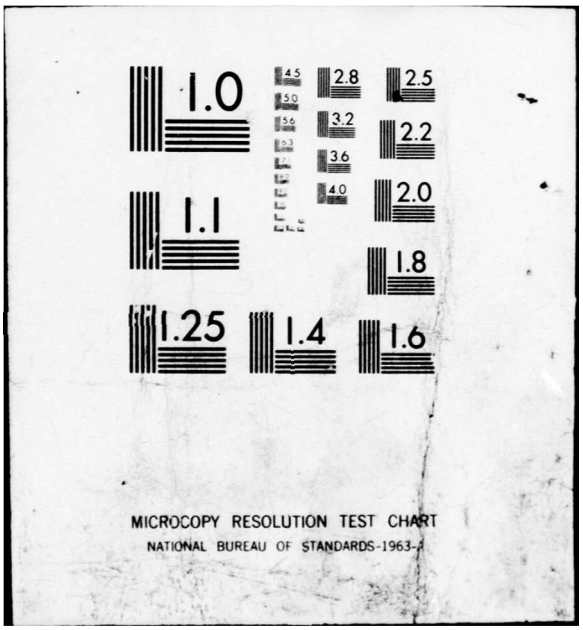
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Models of the dentin tubule system were made with epoxy resin and a low viscosity resin, both used routinely in transmission electron microscopy. Tubular bifurcations and intertubular connections were described using scanning electron microscopy. The significance of the tubular interconnections were discussed.

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THE DENTIN TUBULE SYSTEM : A REPLICA AND SCANNING ELECTRON MICROSCOPE STUDY

John M. Brady

ABSTRACT

Models of the dentin tubule system were made with epoxy resin and a low viscosity resin, both used routinely in transmission electron microscopy. Tubular bifurcations and intertubular connections were described using scanning electron microscopy. The significance of the tubular interconnections were discussed.

Many varieties of resins are available for embedding tissue specimens for transmission electron microscopy. Some of these include Epon 812, Maraglas, Araldites, and a low viscosity resin described by Spurr (Spurr 1969). Dehydration, infiltration and curing techniques with these resins are similar to those described by Luft for Epon 812 (Luft 1961). The universal acceptance of the epoxy resins stems partly from their excellent penetration into soft tissue and their stability in the electron beam. Initial tests with solutions of EDTA and sodium hypochlorite indicated that the cured resins were resistant to these reagents which are used in this laboratory to prepare soft and calcified tissues for scanning and transmission electron microscopy.

The resolution, magnification range and depth of field of the scanning electron microscope and the availability of several viscosities in commercial resins are factors that could be utilized to produce a model of the dentin tubular network. This paper describes the results of two resin preparations to produce dentin tubular casts in human teeth.

MATERIALS AND METHODS

Formalin-fixed human teeth were fractured, immersed in 2.6% sodium hypochlorite in water for 2-15 minute periods in an ultrasonicator and washed for 2 additional 15 minute periods in distilled water. The specimens were then dehydrated in increasing concentrations of alcohol (50, 70, 95 and 100% alcohol/water solutions) followed by propylene oxide (2 x 15 min.), propylene oxide:resin, 1:1 (1 hour), propylene oxide:resin, 1:2 (overnight), resin (4 hours), embedded in fresh resin and cured at 60^{o} C

for 2 days.* Resins were prepared and used according to the instructions supplied with the materials.

After curing, specimens were ground on sandpaper to expose the tooth structure and decalcified in EDTA solution (Warshawsky and Moore 1967) for 2-3 weeks at room temperature with constant stirring. Following decalcification, specimens were again digested in 2.6% sodium hypochlorite for 15 minutes without sonication, washed in distilled water and air-dried overnight. Specimens were mounted on stubs, coated with gold and palladium in a sputterer evaporator and examined on an AMR 1000 scanning electron microscope.**

RESULTS

Epon 812 resin mixture and the low viscosity resin both produced remarkable casts of the dentin tubule system (Fig. 1). At the pulpal border of the casts, the dentin tubules arose from the predentin/dentin interface which in the SEM had the appearance of a series of confluent mounds of calcification encompassing a dozen or more tubules (Fig. 2). After resin penetration and decalcification, the interface was oriented in the SEM to view the dentin/predentin interface from the calcified-dentin aspect, with the dentin tubules emerging into the calcified dentin (Fig. 3). Casts made with the low-viscosity resin produced the finer detail, revealing the intertubular network present at the predentin/dentin interface and throughout the length of the dentin tubule (Figs. 4,5,6). At

*No. 5135 Low Viscosity Embedding Kit and No. 5131 Epon 812 Kit, Ernest F. Fullam, Inc., P.O. Box 444, Schenectady, N. Y. 12301

**Advanced Metals Research Corp, 160 Middlesex Turnpike, Bedford, Mass. 01730

the terminal area of the dentin tubules, a much more dense and elongated intertubular network was evident, with 0.1 - 0.2 μm diameter branches that were up to 15 μm in length (Fig. 6). The high quality of tubular replication can be seen in the micrograph of the epon replica, exhibiting several intertubular connections, originally examined at a magnification of 17,000 times (Fig. 7). In addition to the omnipresent lateral tubule branches, an occasional bifurcated dentinal tubule was evident (Figs. 8 and 3).

DISCUSSION

Results of this study demonstrate that the electron microscopy embedding resins, especially those of low viscosity, produce excellent casts of the dentin tubule system. The anatomy of the uncalcified dentin matrix as visualized herein is remarkable for the anastomoses between dentin tubules and the great number and length of the terminal branches. Patency of these tubules and terminal branches after soft tissue digestion may indicate lack of intratubular calcification even in the most distant branches, and the ease of penetration therein of endodontic reagents from the pulp. Intratubular network throughout the dentin may be a route of intra-dentin extra-cellular fluid circulation, or pathway for spread of toxic agents to tubules at great distance from the primary damaged odontoblast cell processes.

The content of the dentin tubular network that appear in these micrographs is unknown. Odontoblast cell bodies exhibit junctional complexes at several points along adjacent cell membranes. In addition, collagenous, and possibly neural fibrils accompany the odontoblast cell process into

the predentin. Intertubular connections such as described herein are not seen in dentin, fractured, and observed in the SEM. The short circumtubular and intertubular fibers ordinarily observed do not resemble the connections in length or in orientation about the dentin tubule. If these intertubular connections are not cell processes, or remnants of cell-process spaces, the connections may be an array of intertubular collagenous fibers, acting as a scaffold for dentinal tubule array prior to calcification and a route of dentin extracellular circulation after calcification. The possibility of some neural function or content may also be suggested.

CONCLUSION

A technique has been developed to produce a resin cast of the dentin tubular system in human teeth. Casts of the entire dentin tubule have been produced. Examination of the dentin tubular system on the scanning electron microscope reveals bifurcation of occasional tubules. The most notable observation is the presence of intertubular connections, 0.1 - 0.2 μm in diameter between tubules from the predentin/dentin interface to the terminal region of the tubules. At the distal terminals, tubules anastomose long distances, up to 15 μm, in great numbers, and with several tubules. The tubular network may represent a collagenous lattice for tubule organization, a route of dentin extracellular circulation, remnants of odontoblast cell process interconnections or possibly a route of neural structures. This resin cast technique will contribute to a better understanding of dentin structure.

MILITARY DISCLAIMER

Commercial materials and equipment are identified in this report to specify the investigative procedures. Such identification does not imply recommendation or endorsement or that the materials and equipment are necessarily the best available for the purpose. Furthermore, the opinions expressed herein are those of the authors and are not to be construed as those of the U. S. Army Medical Department.

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3. Warshawsky, H. and Moore, G.: A Technique for the Fixation and Decalcification of Rat Incisors for Electron Microscopy. J. Histochem. Cytochem. 15:542, 1967.

Figure Legends

Fig 1. Epon 812 cast of the dentin tubules. Tubules arise from the predentin-dentin interface on the left. Orig. mag. 166X.

Fig 2. SEM of the dentin surface after removal of the predentin. Orig. mag. 3360X.

Fig 3. SEM of an Epon-812 replica of the dentin tubules as they enter the calcified dentin. Orig. mag. 1600X.

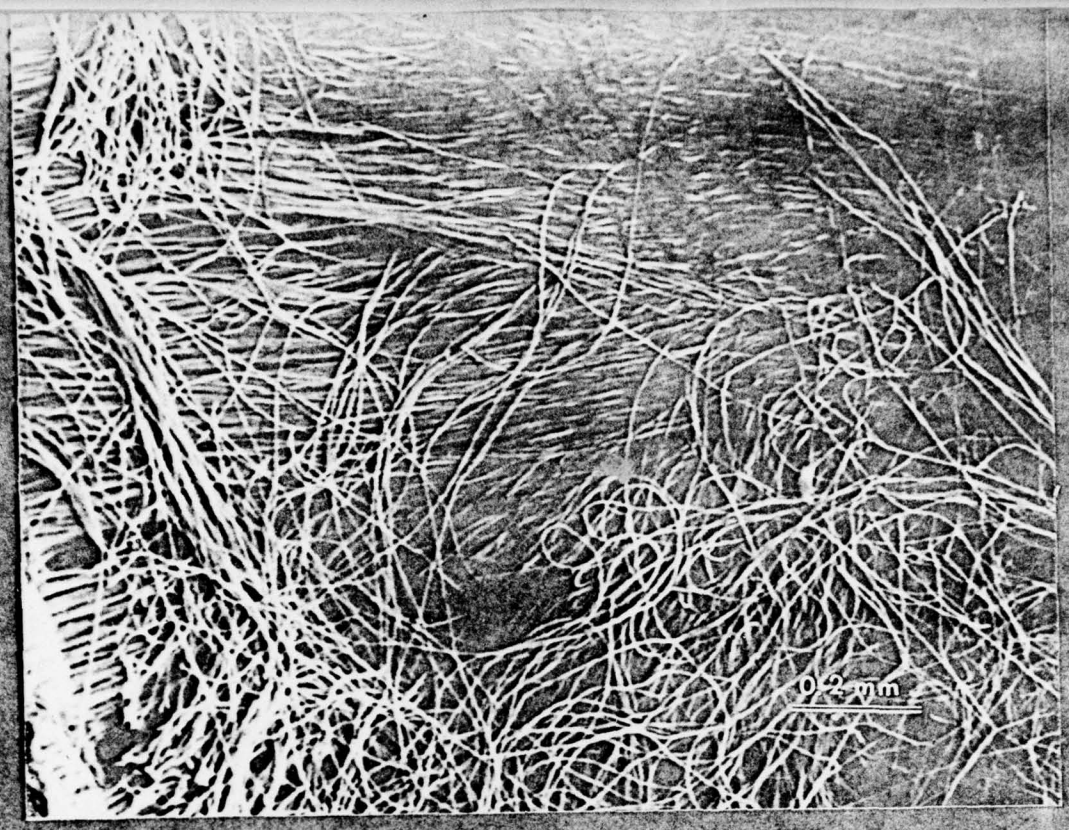
Fig 4. SEM of the low viscosity resin replica of the dentin tubules at the predentin-dentin interface. Tubules are interconnected with many small branches. Orig. mag. 3200X.

Fig 5. SEM of the dentin tubules at mid-point in length. Many small interconnections are evident. Orig. mag. 3040X.

Fig 6. SEM of the dentin tubules near the distal end. A complex intertubular plexus is present. Orig. mag. 7600X.

Fig 7. SEM of the surface of a tubule replica. Several branches are present. Orig. mag. 27,200X.

Fig 8. SEM of dentinal tubules near the predentin border. Occasional bifurcations are present, as shown in the micrograph. Orig. mag. 7200X.



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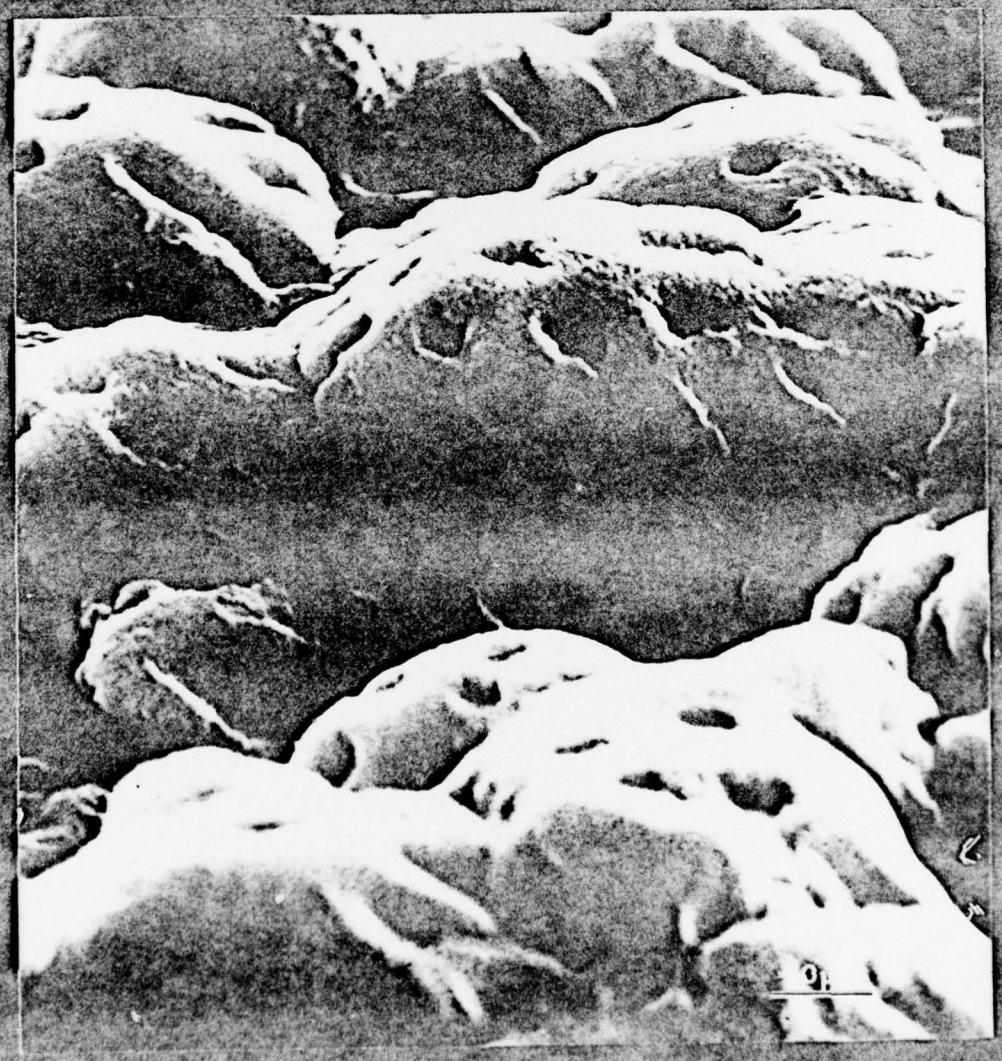

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